THE EPICHLOAE, SYMBIONTS OF THE GRASS SUBFAMILY POÖIDEAE[1]

Christopher L. Schardl[2]

ABSTRACT

Symbioses of grasses (Poaceae) with fungi of family Clavicipitaceae vary widely in relative benefits and detriments to the plant, and include mutualisms characterized by vertical transmission and protective effects of the fungus and its metabolites against vertebrate and invertebrate herbivores. This review focuses on the epichloae, a group of fungi within the Clavicipitaceae that are symbiotic with members of the grass subfamily Poöideae and comprise sexual fungi of genus *Epichloë* (Fr.) Tul. & C. Tul. and their asexual derivatives (form genus *Neotyphodium* A. E. Glenn, C. W. Bacon & R. T. Hanlin). Most epichloid fungi are vertically transmissible, and many produce antiherbivore alkaloids belonging to any of four distinct chemical classes: lolines, peramine, ergot alkaloids, and indole-diterpenes. Like many plant-associated Clavicipitaceae, fruiting of *Epichloë* species chokes host inflorescences, preventing seed production on the affected tillers. However, most *Epichloë*-infected grasses also produce asymptomatic inflorescences, which produce normal seeds that vertically transmit the symbiont. Vertical transmission provides the main or sole means of dissemination for asexual epichloae. Molecular phylogenetic evidence suggests that the origin of Poöideae-*Epichloë* symbioses was approximately coincident with the origin of this grass subfamily, with considerable cophylogenetic evolution since then. Most asexual epichloae arose from a more complex process of interspecific hybridizations, which can provide evolutionary diversification and counteract accumulated deleterious mutations in otherwise clonal symbionts. Evolution of the Poöideae-epichloae symbioses required a break in the link between virulence and transmission, by establishing highly vertical transmission without negative effects on the colonized seeds and seedlings.

Key words: Alkaloids, bioprotection, Clavicipitaceae, coevolution, cophylogeny, *Epichloë*, Hypocreales, mutualism, *Neotyphodium*, Poaceae, Poöideae, symbiosis.

Among the mechanisms that plants deploy against herbivores, chemical defenses are widespread, varied, and important (Schardl & Chen, 2010). However, plants do not produce all of their own defensive metabolites; some are produced by plant symbionts (Bush et al., 1997; Markert et al., 2008; Ralphs et al., 2008). Among these are fungi in the genus *Epichloë* (Fr.) Tul. & C. Tul. (order Hypocreales) and their asexual derivatives in form genus *Neotyphodium* A. E. Glenn, C. W. Bacon & R. T. Hanlin, which form symbioses with grasses (Poaceae) in the subfamily Poöideae (Schardl et al., 2008) (Table 1). For convenience, and in recognition of the close relationship of *Epichloë* and *Neotyphodium* species, I refer to these fungi collectively as epichloae (adjective, epichloid). The Poöideae-epichloae symbioses are systemic, in that the fungi are present in most aboveground host tissues (but with little or no colonization of roots). Furthermore, they are constitutive symbioses because they are maintained throughout the life of the host plant. Most *Epichloë* species and all asexual *Neotyphodium* species are vertically transmissible. The vertical transmission process is especially interesting because it involves extensive colonization of the host ovary, ovule, and embryo, without causing any damage or symptoms (Freeman, 1904).

Although vertical transmission via seeds is an important dissemination mechanism for most epichloae, in the sexual state they cause a replacement disease called "choke," whereby they actually suppress maturation and prevent seed production on the affected inflorescences. Choked culms instead manifest ectophytic proliferation of the fungus to form stromata bearing flask-shaped fruiting structures (perithecia), within which meiosis culminates in ascospore production (Fig. 1). Ascospores then mediate infections of new host plants or seeds on neighboring plants (Chung & Schardl, 1997a; Brem & Leuchtmann, 1999). Most host-*Epichloë* symbiota exhibit choke disease on only some of the flowering tillers, while the other tillers bear the same fungal genet in a completely asymptomatic association. Thus, the interaction of a single host individual with a single *Epichloë* species individual simultaneously exhibits

---

[1] I am grateful for reviews by Victoria C. Hollowell and Peter F. Stevens (Missouri Botanical Garden), Barry Scott (Massey University, Palmerston, New Zealand), and Adrian Leuchtmann (ETH, Zürich, Switzerland). *Epichloë typhina* population surveys and DNA sequence analysis were supported by the ETH, Zürich, Switzerland, and conducted in the laboratories of Bruce A. McDonald and Adrian Leuchtmann. This is publication number 10-12-094 of the Kentucky Agricultural Experiment Station, published with approval of the director.

[2] Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546-0312, U.S.A. schardl@uky.edu.

doi: 10.3417/2009144

ANN. MISSOURI BOT. GARD. 97: 646–665. PUBLISHED ON 27 DECEMBER 2010.

very different phenotypes on different tillers, allowing for both horizontal transmission of sexually derived spores and vertical transmission of clonally derived hyphae (Sampson, 1933).

Vertical transmissibility is one of the characteristics of the epichloae that predisposes them to evolve into forms that are strongly beneficial to the host. In particular, the asexual derivatives (*Neotyphodium*) cause no choke disease (with occasional exceptions) and are entirely dependent on vertical transmission for their dissemination. Significant host benefits, particularly against insect herbivory, have been documented for *N. coenophialum* (Morgan-Jones & W. Gams) A. E. Glenn, C. W. Bacon & R. T. Hanlin (cf. Table 1 for attributions and isolates of epichloae) in *Lolium arundinaceum* (Schreb.) Darbysh. [≡ *Schedonorus arundinaceus* (Schreb.) Dumort., ≡ *Festuca arundinacea* Schreb.], *N. lolii* (Latch, M. J. Chr. & Samuels) A. E. Glenn, C. W. Bacon & R. T. Hanlin in *L. perenne* L., *N. uncinatum* (W. Gams, Petrini & D. Schmidt) A. E. Glenn, C. W. Bacon & R. T. Hanlin in *L. pratense* (Huds.) Darbysh. [≡ *S. pratensis* (Huds.) P. Beauv., ≡ *F. pratensis* Huds.], and a symbiont likely to be *Epichloë festucae* Leuchtm., Schardl & M. R. Siegel in *F. rubra* L. (Clay et al., 1993; Wilkinson et al., 2000; Tanaka et al., 2005). Enhanced drought tolerance is another documented characteristic of *N. coenophialum* (Malinowski & Belesky, 2000), as well as *N. uncinatum* in *L. pratense* (Malinowski et al., 1997). Reported effects of *N. lolii* on drought tolerance of *L. perenne* are more variable, probably due to selection for adaptation to different habitats (Hesse et al., 2005). Antinematode activity and several additional host benefits have also been documented in the *Lolium arundinaceum–Neotyphodium coenophialum* system (Malinowski & Belesky, 2000; Timper et al., 2005). These symbioses, where the fungus is mainly or exclusively disseminated by systemically colonizing host seeds, fit well with the prediction that vertical transmission selects for mutualism (Ewald, 1987).

EPICHLOID ALKALOIDS AND HOST PROTECTION

Several classes of alkaloids produced by epichloae (Fig. 2) deter or kill insects and, in some cases, vertebrate herbivores. Protective roles of lolines and peramine against insects have been demonstrated by genetic and molecular genetic tests, respectively (Wilkinson et al., 2000; Tanaka et al., 2005). Other epichloid alkaloids act against both mammalian grazers and insects. A recent genetic test demonstrated that ergot alkaloids contribute antifeedant and toxic effects against the insect *Agrotis ipsilon* (Potter et al., 2008). In mammalian systems, neurotropic activities of the ergot alkaloids in general, and ergopeptines in particular, have been known for a very long time (reviewed in Schardl et al., 2006). Likewise, the toxic effects of the indole-diterpenes, also referred to as tremorgens, are well established (Knaus et al., 1994; Parker & Scott, 2004). The antimammalian alkaloids produced by epichloae symbiotic with temperate forage grasses cause significant losses to livestock productivity. This economic impact has fueled intensive research into the roles and effects of epichloae in cultivated forage grasses as in *Lolium* L. and *Festuca* L. The claim that the protective effects have arisen by selective breeding (Faeth, 2002) ignores the fact that potent antiherbivore alkaloids and anti-insect activities characterize wild populations of these grasses (Christensen et al., 1993), as well as uncultivated grasses such as *Agrostis hyemalis* (Walter) Britton, Sterns & Poggenb., *Achnatherum inebrians* (Hance) Keng, *Bromus benekenii* (Lange) Trimen, *Echinopogon ovatus* (G. Forst.) P. Beauv., *Glyceria striata* (Lam.) Hitchc., *Poa autumnalis* Muhl. ex Elliot, and others (Cheplick & Clay, 1988; Leuchtmann et al., 2000; Schardl et al., 2006, 2007; Gonthier et al., 2008).

The epichloae have been shown to affect food web structure (Omacini et al., 2001) and ecological succession. A long-term study has compared plant succession in stands of *Lolium arundinaceum* with high or low frequencies of *Neotyphodium coenophialum* infection (Clay et al., 2005). The highly infested plots showed considerably less plant diversity and dramatically suppressed succession to woody plants compared to those with low infection frequency. Experimental exclusion of mammals and insects indicated that both had a role in this phenomenon. Voles in particular would tend to eat the grass in preference to woody plants unless the grass was infected with the endophyte. This endophyte produces lolines and peramine, neither of which appears to deter mammals, and ergot alkaloids (Siegel et al., 1990) (Fig. 2). In a genetic experiment with a ryegrass endophyte, ergot alkaloids appeared to be a significant deterrent to feeding by rabbits (Panaccione et al., 2006a). Furthermore, grasses with very high levels of ergonovine and lysergic acid amide appear to deter grazing by livestock. The common names of these grasses, drunken horse grass (*Achnatherum inebrians*) and sleepy grass (*A. robustum* (Vasey) Barkworth), reflect this situation. After naive animals eat the grass and suffer stupor due to the ergot alkaloids, they avoid those grasses thereafter. It is the endophytes of these grasses that produce the deterrent ergot alkaloids (Petroski et al., 1992; Miles et al., 1996).

Some epichloae also impart or induce potent antinematode activities, although it is unclear if any

Table 1. Relationships of *Epichloë* and *Neotyphodium* species. Multiple clades are indicated for interspecific hybrids (cf. Fig. 4).

Fungal species[1]	Host grass species	Host tribe	Host geographic origin[2]	Clade relationships[3]
Epichloë amarillans J. F. White	*Agrostis* L. spp., *Sphenopholis* Scribn. spp.	Poeae	N. America	Eam
E. baconii J. F. White	*Agrostis* spp., *Calamagrostis villosa* J. F. Gmel.	Poeae	Europe	Eba
E. brachyelytri Schardl & Leuchtm.	*Brachyelytrum erectum* (Schreb.) P. Beauv.	Brachyelytreae	N. America	Ebe
E. bromicola Leuchtm. & Schardl	*Bromus benekenii* (Lange) Trimen, *B. erectus* Huds., *B. ramosus* Huds.	Bromeae	Europe	EbcC
E. bromicola	*Hordelymus europaeus* (L.) Harz	Triticeae	Europe	EbcC
E. clarkii J. F. White	*Holcus lanatus* L.	Poeae	Europe	EtC
E. elymi Schardl & Leuchtm.	*Bromus kalmii* A. Gray	Bromeae	N. America	Eel
E. elymi	*Elymus* L. spp.	Triticeae	N. America	Eel
E. festucae Leuchtm., Schardl & M. R. Siegel	*Festuca* L. spp., *Lolium* L. spp.	Poeae	Europe	Efe
E. festucae	*Koeleria pyramidata* (Lam.) P. Beauv.	Poeae	Europe	Efe
E. glyceriae Schardl & Leuchtm.	*Glyceria striata* (Lam.) Hitchc.	Meliceae	N. America	Egl
E. sylvatica Leuchtm. & Schardl	*Brachypodium sylvaticum* (Huds.) P. Beauv.	Brachypodieae	Europe, Asia	EtC
E. typhina (Fr.) Tul. & C. Tul.	*Anthoxanthum odoratum* L., *Dactylis glomerata* L., *Lolium perenne* L., *Poa nemoralis* L., *P. pratensis* L., *P. sylvicola* Guss., *P. trivialis* L., *Puccinellia distans* (Jacq.) Parl.	Poeae	Europe	EtC
E. typhina	*Brachypodium pinnatum* (L.) P. Beauv.	Brachypodieae	Europe	EtC
E. typhina	*Phleum pratense* L.	Poeae	Europe	EtC
E. yangzii Wei Li & Zhi Wei Wang	*Roegneria kamoji* (Ohwi) Keng & S. L. Chen	Bromeae	Asia	EbcC
Epichloë sp.	*Holcus mollis* L.	Poeae	Europe	EHm
Neotyphodium aotearoae C. D. Moon, C. O. Miles & Schardl	*Echinopogon ovatus* (G. Forst.) P. Beauv.	Poeae	Australia, New Zealand	Nao
N. australiense C. D. Moon & Schardl	*Echinopogon ovatus*	Poeae	Australia	Efe, EtC
N. chisosum (J. F. White & Morgan-Jones) A. E. Glenn, C. W. Bacon & R. T. Hanlin	*Achnatherum eminens* (Cav.) Barkworth	Stipeae	N. America	Eam, EbcC, EtC
N. coenophialum (Morgan-Jones & W. Gams) A. E. Glenn, C. W. Bacon & R. T. Hanlin	*Lolium arundinaceum* (Schreb.) Darbysh. [≡ *Schedonorus arundinaceus* (Schreb.) Dumort., ≡ *Festuca arundinacea* Schreb.]	Poeae	Europe, N. Africa	Efe, EtC, Eba
N. funkii K. D. Craven & Schardl	*Achnatherum robustum* (Vasey) Barkworth	Stipeae	N. America	Eel, Efe
N. gansuense C. J. Li & Nan	*Achnatherum inebrians* (Hance) Keng	Stipeae	Asia	NgC
N. guerinii J. J. Gauillomin, C. Ravel & C. D. Moon	*Melica ciliata* L.	Meliceae	N. Africa	EtC, NgC
N. huerfanum (J. F. White, G. T. Cole & Morgan-Jones) A. E. Glenn, C. W. Bacon & R. T. Hanlin	*Festuca arizonica* Vasey	Poeae	N. America	EtC
N. lolii (Latch, M. J. Chr. & Samuels) A. E. Glenn, C. W. Bacon & R. T. Hanlin	*Lolium perenne*	Poeae	N. Africa	Efe
N. lolii × *E. typhina* sp. indet., LpTG-2	*Lolium perenne*	Poeae	Europe	Efe, EtC
N. melicicola C. D. Moon & Schardl	*Melica decumbens* Thunb.	Meliceae	S. Africa	Efe, Nao

Table 1. Continued.

Fungal species[1]	Host grass species	Host tribe	Host geographic origin[2]	Clade relationships[3]
N. occultans C. D. Moon, B. Scott & M. J. Chr.	*Lolium*, annual species	Poeae	N. Africa	EbcC, Eba
N. pampeanum Iannone & Cabral	*Bromus auleticus* Trin. ex Nees	Bromeae	S. America	Efe, EtC
N. siegelii K. D. Craven, Leuchtm. & Schardl	*Lolium pratense* (Huds.) Darbysh. [≡ *Schedonorus pratensis* (Huds.) P. Beauv., ≡ *Festuca pratensis* Huds.]	Poeae	N. Africa	EbcC, Efe
N. sinicum Z. W. Wang, Y. L. Ji & Y. Kang	*Roegneria* K. Koch spp.	Triticeae	Asia	EbcC, EtC
N. sinofestucae Y. Chen, Y. Ji & Z. W. Wang	*Festuca parvigluma* Steud.	Poeae	Asia	EbcC, EtC
N. stromatolongum Y. L. Ji, L. H. Zhan & Z. W. Wang	*Calamagrostis epigejos* (L.) Roth	Poeae	Asia	Nst
N. tembladerae Cabral & J. F. White	*Festuca arizonica*	Poeae	N. America	Efe, EtC
N. tembladerae	*Bromus auleticus*, *B. setifolius* J. Presl	Bromeae	S. America	Efe, EtC
N. tembladerae	*Festuca argentina* (Speg.) Parodi, *F. hieronymi* Hack., *F. magellanica* Lam., *F. superba* Parodi ex Türpe, *Poa huecu* Parodi, *P. rigidifolia* Steud.	Poeae	S. America	Efe, EtC
N. tembladerae	*Melica stuckertii* Hack.	Meliceae	S. America	Efe, EtC
N. tembladerae	*Phleum commutatum* Gaudin	Poeae	S. America	Efe, EtC
N. typhinum (Morgan-Jones & W. Gams) A. E. Glenn, C. W. Bacon & R. T. Hanlin	*Poa ampla* Merr., *P. sylvestris* A. Gray	Poeae	N. America	EtC
N. typhinum var. *canariense* C. D. Moon, B. Scott & M. J. Chr.	*Lolium edwardii* H. Scholz, Stierst. & Gaisberg	Poeae	Europe	EtC
N. uncinatum (W. Gams, Petrini & D. Schmidt) A. E. Glenn, C. W. Bacon & R. T. Hanlin	*Lolium pratense*	Poeae	Europe	EbcC, EtC
N. sp. indet., FaTG-2	*Lolium* sp.	Poeae	Europe, N. Africa	Efe, Eba
N. sp. indet., FaTG-3	*Lolium* sp.	Poeae	Europe, N. Africa	EtC, Eba
N. sp. indet., FalTG-1	*Festuca altissima* All.	Poeae	Europe	EbcC, EtC
N. sp. indet., FobTG-1	*Festuca obtusa* (Pers.) E. B. Alexeev	Poeae	N. America	Efe
N. sp. indet., FpaTG-1	*Festuca paradoxa* Desv.	Poeae	N. America	Eam, EtC
N. sp. indet., HeuTG-2	?*Hordelymus europaeus*	Triticeae	Europe	EbcC, EtC
N. sp. indet., HboTG-1	*Hordeum bogdanii* Wilensky	Triticeae	Europe	Eam, Eel
N. sp. indet., HboTG-2	*Hordeum bogdanii*	Triticeae	Europe	EbcC, EtC
N. sp. indet., HbrTG-1	*Hordeum brevisubulatum* (Trin.) Link	Triticeae	Europe	EbcC
N. sp. indet., HbrTG-2	*Hordeum brevisubulatum*	Triticeae	Europe	EbcC, EtC
N. sp. indet., PauTG-1	*Poa autumnalis* Muhl. ex Elliot	Poeae	N. America	Eel, EtC
N. sp. indet., PhcTG-2	*Phleum commutatum*	Poeae	S. America	Efe, Eba, Eam

[1] Undescribed species of *Epichloë* and *Neotyphodium* are designated as taxonomic groupings (TG) by the grass taxon for first isolates, according to the convention of Christensen et al. (1993).

[2] Following classification scheme of Barkworth et al. (2007).

[3] Major clades are identified by their described nonhybrid species, abbreviated as follows: Eam, *E. amarillans*; Eba, *E. baconii*; EbcC, *E. bromicola* complex; Ebe, *E. brachyelytri*; Eel, *E. elymi*; Efe, *E. festucae*; Egl, *E. glyceriae*; EHm, *Epichloë* sp. indet. from *Holcus mollis*; EtC, *E. typhina* complex; Nao, *N. aotearoae*; NgC, *N. gansuense* clade; Nst, *N. stromatolongum*.

of the alkaloids contribute. In a study of an endophyte of perennial ryegrass and its effect against the root-parasitic nematode *Pratylenchus scribneri*, ergot alkaloids were ruled out as the main factors (Panaccione et al., 2006b), and lolines were not expressed in that system. Other studies show that lolines can have affects on nematodes, both as attractants at lower concentrations, and as toxins at

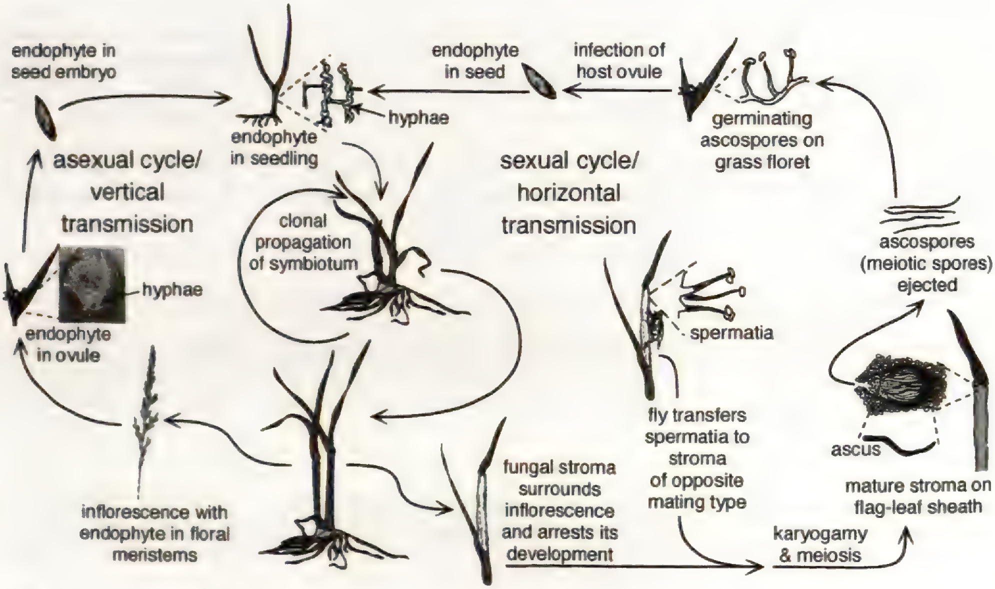
endophyte in seed embryo
endophyte in seedling
hyphae
endophyte in seed
infection of host ovule
germinating ascospores on grass floret
asexual cycle/ vertical transmission
clonal propagation of symbiotum
sexual cycle/ horizontal transmission
ascospores (meiotic spores) ejected
hyphae
endophyte in ovule
spermatia
fly transfers spermatia to stroma of opposite mating type
ascus
mature stroma on flag-leaf sheath
inflorescence with endophyte in floral meristems
fungal stroma surrounds inflorescence and arrests its development
karyogamy & meiosis

Figure 1. Life cycles of *Epichloë* species in relationship to the life cycles of their host grasses. Some *Epichloë* species simultaneously undergo sexual and asexual life cycles on different tillers of infected host grasses, as shown here. Obligately sexual *Epichloë* species transmit only horizontally and tend to suppress almost all seed production by the infected hosts. Obligately asexual epichloae (*Neotyphodium* species) fail to produce stromata and are vertically transmitted. The micrograph at left shows an ovule infected with *E. festucae* expressing a gene for cyan fluorescent protein.

higher concentrations (Bacetty et al., 2009). Whether these effects are relevant to plant-nematode interactions in the field remains to be determined. Another intriguing possibility is the effect of lolines on foliar nematodes. The common endophyte of annual ryegrasses is *Neotyphodium occultans* C. D. Moon, B. Scott & M. J. Chr., and plants with this endophyte usually have lolines (TePaske et al., 1993). Annual ryegrass forage (e.g., *Lolium rigidum* Gaudin and *L. multiflorum* Lam.) can sometimes be toxic to livestock when infected with *Clavibacter toxicus*, which is in turn infected with a bacteriophage believed to encode a toxin (McKay & Ophel, 1993). The bacterium is vectored by the foliar nematode *Anguina funesta*. It is unknown whether the ryegrass stands that caused toxicosis possessed or lacked the endophyte. Conceivably, the fact that annual ryegrass toxicosis is a rare phenomenon may relate at least partly to antinematode activity of this endophyte alkaloid. Thus, the effects of the alkaloids on foliar nematodes would be interesting to investigate.

Among the species of epichloae, there is considerable diversity in the alkaloid profiles, and even diversity within some species. For example, *Epichloë festucae*, which has been studied more extensively at this level than have any of the other sexual species, includes strains that produce various combinations of all four classes of alkaloids (Schardl, 2001) (Table 2). The chemotypic diversity of *E. festucae* is particularly interesting considering that phylogenetic analysis based on intron sequences of housekeeping genes indicates exceptionally low sequence variation for this species (Moon et al., 2004). In contrast, *E. typhina* (Fr.) Tul. & C. Tul. has much greater sequence diversity, but very little chemotypic variation: no loline-, indole-diterpene–, or ergot-alkaloid–producing strains of *E. typhina* or its close relatives, *E. sylvatica* Leuchtm. & Schardl and *E. clarkii* J. F. White, having been identified to date (Leuchtmann et al., 2000; Young et al., 2009). Anti-insect activity attributed to strains of *E. sylvatica* indicates that additional fungal metabolites or protective effects on host plants remain to be identified (Brem & Leuchtmann, 2001).

RELATIONSHIPS AMONG PLANT-ASSOCIATED CLAVICIPITACEAE

Although the fungal order Hypocreales is dominated by plant parasites and symbionts, the family Clavicipitaceae s.l. comprises primarily insect parasites. Recently, this family has been reassessed based on multiple gene sequences, erecting the families Cordycipitaceae and Ophiocordycipitaceae (Sung et al., 2007), both of which are mainly insect parasites.

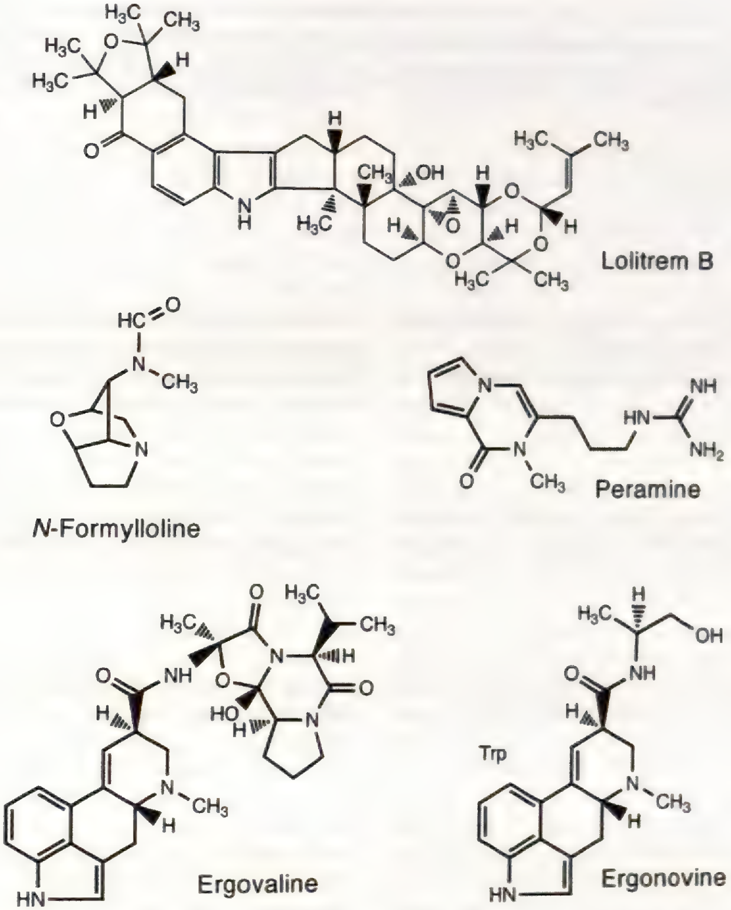

Figure 2. Examples of alkaloids produced by epichloae. Lolitrem B is the most abundant indole diterpene produced by *Neotyphodium lolii*, a symbiont of *Lolium perenne*. *N*-Formylloline is the predominant loline alkaloid produced by most strains of *N. coenophialum* in *L. arundinaceum*. Peramine is a pyrrolopyrazine alkaloid produced by many *Epichloë* and *Neotyphodium* species. Ergovaline is the predominant ergopeptine produced by, for example, *N. coenophialum* and *N. lolii*, whereas the simpler ergot alkaloid, ergonovine, is produced by *N. gansuense* in *Achnatherum inebrians* (drunken horse grass) as a major deterrent to grazers.

Clavicipitaceae s. str. includes insect parasites (such as *Hypocrella* and *Metarhizium* spp.) as well as a clade of plant parasites and plant symbionts. These plant-associated Clavicipitaceae include the genera *Claviceps* Tul., *Balansia* Speg., *Epichloë*, and *Myriogenospora* G. F. Atk. Based on phylogenetic studies of ribosomal RNA genes (rDNA) (Kuldau et al., 1997; White & Reddy, 1998; Sullivan et al., 2001) and the gene for acetaldehyde dehydrogenase ALDH1-1 (Tanaka & Tanaka, 2008), this clade probably also includes the genera *Aciculosporium* I. Miyake, *Atkinsonella* Diehl, *Balansiopsis* Höhn, *Heteroepichloë* E. Tanaka, C. Tanaka, Gafur & Tsuda, *Neoclaviceps* J. F. White, Bills, S. C. Alderman & Spatafora, *Parepichloë* J. F. White & P. V. Reddy, and *Ustilaginoidea* Bref. Most of the plant-associated Clavicipitaceae infect Poaceae (Diehl, 1950; Bischoff & White, 2003). Some infect sedges (Cyperaceae), and a clavicipitaceous, seed-transmissible symbiont of *Ipomoea* L. and *Turbina* Raf. species (Convolvulaceae) has recently been identified (though not yet described and named) (Steiner et al., 2006). With the exception of the *Claviceps* and perhaps *Neoclaviceps* species, these plant-associated Clavicipitaceae tend to have constitutive symbioses with their host plants. Specifically, they grow endophytically within, or epibiotically upon, the aerial tissues of the plant, without obvious signs or symptoms in most of the colonized tissues. Their presence is conspicuous only by their fruiting bodies (stromata and sclerotia), which form on specific organs or locations such as the florets,

Table 2. Alkaloid profiles of grass species symbiotic with *Epichloë festucae*. Data are from Siegel et al. (1990).

Host	Lolines	Peramine	Ergot alkaloids	Indole-diterpenes
Lolium giganteum (L.) Darbysh.	+	+	−	−
Festuca ovina L.	−	+	+	−
F. longifolia Thuill.	−	+	+	+
F. rubra L. subsp. *rubra*	−	−	−	+
F. rubra subsp. *rubra*	−	−	+	−
F. rubra subsp. *commutata* Gaudin	−	+	+	−
F. rubra subsp. *commutata*	−	+	−	−

inflorescences, nodes, or segments of the leaves (Diehl, 1950; Bischoff & White, 2003).

More extensive study is required to determine if the plant-symbiotic Clavicipitaceae, together with *Claviceps* and *Neoclaviceps* species, constitute a monophyletic clade. In addition to more comprehensive phylogenetic analysis, further characterization of host interactions is needed for several related Clavicipitaceae that are found fruiting on plants; e.g., *Dussiella tuberiformis* (Berk. & Ravenel) Pat. on *Arundinaria tecta* (Walter) Muhl. (Poaceae), *Shimizuomyces paradoxus* Kobayasi on fruit of *Smilax sieboldii* Miq. (Smilacaceae), and several other genera of plant-associated Clavicipitaceae (Bischoff & White, 2003). Some of these may first infect sucking insects on the plant and then access plant nutrients via the insect stylet. Such appears to be the case for *Hyperdermium bertonii* (Speg.) J. F. White, R. F. Sullivan, Bills & Hywel-Jones, found in association with a scale insect on an unidentified Asteraceae (Sullivan et al., 2000). This observation fuels speculation that parasitism of plant-associated invertebrates provided an evolutionary link to plant-symbiotic fungi in this family (Sullivan et al., 2000; Spatafora et al., 2007).

Effects of the clavicipitaceous fungi on their host plants suggest that they alter the balance of plant growth regulators. *Aciculosporium take* I. Miyake, *Heteroepichloë sasae* (Hara) E. Tanaka, C. Tanaka, Gafur & Tsuda, and *H. bambusae* (Pat.) E. Tanaka, C. Tanaka, Gafur & Tsuda cause witches' broom of bamboos (Tanaka et al., 2002; Tanaka & Tanaka, 2008). Many of the other fungi suppress host flowering. As described above, *Epichloë* species can fruit on immature inflorescences and halt their further maturation. *Balansia epichloë* (Weese) Diehl, *B. henningsiana* (Möller) Diehl, and *Myriogenospora atramentosa* (Berk. & M. A. Curtis) Diehl form their stromata on leaves, but nevertheless also partially or completely suppress host flowering (Clay et al., 1989; Glenn et al., 1998). Thus, these plant parasites alter development in a way that appears to redirect resources away from host seed production to support fungal sporulation.

Many fungal species allied with the Clavicipitaceae lack a known sexual state, either because they never form stromata or, more rarely, because their stromata have not been observed to progress past the production of mitotic spores (conidia) to produce meiotic spores. These fungi are described as either *Neotyphodium* or *Ephelis* Fr. species depending on the ontogeny and morphology of their conidia. Use of these genus names conforms to the *International Code of Botanical Nomenclature* (cf. Art. 59; McNeill et al., 2006), requiring that any fungi of phyla Ascomycota and Basidiomycota that lack a known sexual state (teleomorph) be given distinct form names that describe the asexual state (anamorph). Arguably, the availability of facile molecular techniques to establish relationships of isolates should obviate the need for this arcane system, but the requirement remains entrenched in the Code. The implication for the plant-associated Clavicipitaceae is that those fungi classified in form genus *Neotyphodium* are (with the exception of *Acremonium chilense* J. F. White & Morgan-Jones, ≡ *N. chilense* (J. F. White & Morgan-Jones) A. E. Glenn, C. W. Bacon & R. T. Hanlin) molecular congeners of *Epichloë* species (Glenn et al., 1996). Also, the *Ephelis* anamorph that is usually associated with *Balansia* and related genera has also been described without an associated teleomorph (Christensen et al., 2000b). Interestingly, *Atkinsonella* species actually produce two mitotic spore states, one resembling the *Neotyphodium* anamorph, and the other resembling the *Ephelis* anamorph (Leuchtmann & Clay, 1988).

EPICHLOË SPECIES

The *Epichloë* species are members of the fungal phylum Ascomycota, order Hypocreales, and are characterized by systemic, intercellular growth in a host grass (Poaceae) and production of a brightly colored stroma (fruiting body) that envelops the host leaf sheath and subtending immature inflorescence of the young flowering culm (Ellis & Everhart, 1886). Upon emergence, the initially unpigmented stroma produces palisades of conidiogenous cells giving rise to conidia that serve as spermatia. Cross-fertilization is most often mediated by female dipteran flies of genus *Botanophila* Lioy. (Anthomyiidae), which lay one to several eggs on each stroma, and for which the maturing stroma provides nutrition for their larvae (Bultman et al., 1998). Fertilized stromata mature to form flask-shaped perithecia within which spore sacs (asci) develop. Each ascus bears eight meiotically derived spores (ascospores), which are filamentous and contain numerous haploid nuclei. In some species, these spores tend to break up into part-spores on maturation (White, 1993). As perithecia mature, they accumulate yellow to orange carotenoid pigments. This bright coloration is distinct from the gray, dark brown, or black pigmentation in stromata or sclerotia (resting structures) of most other plant-associated Clavicipitaceae. Mature ascospores are ejected through the apical pore of the ascus and the ostiole of the perithecium, and mediate infection of new plants or developing seeds on neighboring plants (Chung & Schardl, 1997a).

HOST SPECIFICITY

As currently defined, and consistent with many molecular phylogenetic analyses (Kuldau et al., 1997; Bischoff & White, 2003; Sung et al., 2007; Tanaka & Tanaka, 2008), *Epichloë* species and *Neotyphodium* species (asexual descendants of *Epichloë* species) together form a monophyletic group. Interestingly, their hosts are all in subfamily Poöideae, also a monophyletic group (Soreng & Davis, 1998; Kellogg, 2001). Host species have been identified in most tribes of the Poöideae. The only other Clavicipitaceae known to infect pooid grasses are some *Claviceps* species and *Balansia texensis* (Diehl) P. V. Reddy, Clay & J. F. White [≡ *Atkinsonella texensis* (Diehl) Leuchtm. & Clay], which systemically infects and chokes inflorescences on *Nassella leucotricha* (Trin. & Rupr.) R. W. Pohl (Morgan-Jones & White, 1989).

Although *Epichloë* species are found in a broad phylogenetic range of the Poöideae, most described species of *Epichloë* are apparently restricted to a single host genus or closely related genera within a tribe (Table 1). However, the possibility must be considered that species are sometimes described on the assumption of restricted host range. Therefore, molecular phylogenetic analysis and mating tests are important to circumscribe species. Most *Epichloë* species represent populations of interfertile genotypes, and phylogenetic analyses of intron sequences in genes for β-tubulin (*tubB*), γ-actin (*actG*), and translation elongation factor 1-α (*tefA*) (Fig. 3) indicate that most described *Epichloë* species correspond to unique clades (ignoring the asexual species and groups, which I discuss later). Thus, there is a close correspondence between host range, interfertility, and phylogenetic relationships of *E. amarillans* J. F. White, *E. brachyelytri* Schardl & Leuchtm., *E. bromicola* Leuchtm. & Schardl, *E. elymi* Schardl & Leuchtm., *E. glyceriae* Schardl & Leuchtm., and *E. sylvatica*.

Other relationships are intriguingly more complex (Fig. 3). *Epichloë festucae* constitutes a clade with extremely low sequence variation, and mainly associated with *Festuca* and *Lolium* species (Leuchtmann et al., 1994), though also found on *Koeleria pyramidata* (Lam.) P. Beauv. (originally reported as *K. cristata* Pers., nom. illeg.; Craven et al., 2001b). Chloroplast DNA phylogeny places *K. pyramidata* on a well-supported clade separate from the *Festuca–Lolium* clade, in contrast to the close sequence relationship of the *E. festucae* isolates from those hosts (Schardl et al., 2008). Interestingly, attempted matings between an isolate from *K. pyramidata* and *E. festucae* stromata on *Festuca rubra* failed to yield viable ascospores (Craven et al., 2001b). This situation is not unique. Many isolates of *E. typhina* on *Brachypodium pinnatum* (L.) P. Beauv. (tribe Brachypodieae) appear to be very close phylogenetically to *E. sylvatica*, which has only been identified on *B. sylvaticum* (Huds.) P. Beauv. (Fig. 3). However, test matings indicate that *E. sylvatica* is incompatible with *E. typhina* on *B. pinnatum* (Leuchtmann & Schardl, 1998). In contrast, *E. typhina* isolates from several host genera and tribes are interfertile. These findings illustrate that prezygotic isolation is not simply related to degree of phylogenetic divergence, and that closely related strains from different hosts sometimes have strong prezygotic isolation.

As currently circumscribed, *Epichloë typhina* has a broad host range including several grass genera in tribe Poeae, as well as in *Brachypodium pinnatum* (Brachypodieae). However, a population genetic study suggests that *E. typhina* is a complex of genetically isolated populations associated with different hosts (Schardl et al., unpublished). Isolates were surveyed from five host species in Europe: *B. pinnatum*, *Dactylis glomerata* L., *Poa trivialis* L., *Poa nemoralis* L., and *Puccinellia distans* (Jacq.) Parl. Sequences were obtained from the intron-rich 5′-segments of *tubB* and *tefA* genes (Figs. 3, 4). None of the haplotypes were found in association with more than one host, indicating strong genetic isolation between host-specific groups. The possibility of similar host-based subdivisions of *Epichloë* species remains to be investigated in the cases of *E. baconii* J. F. White and *E. amarillans*. Possible underlying mechanisms could include host phenology (particularly flowering time), genetic determinants of host specificity, or specific preferences of *Botanophila* flies for stromata on each

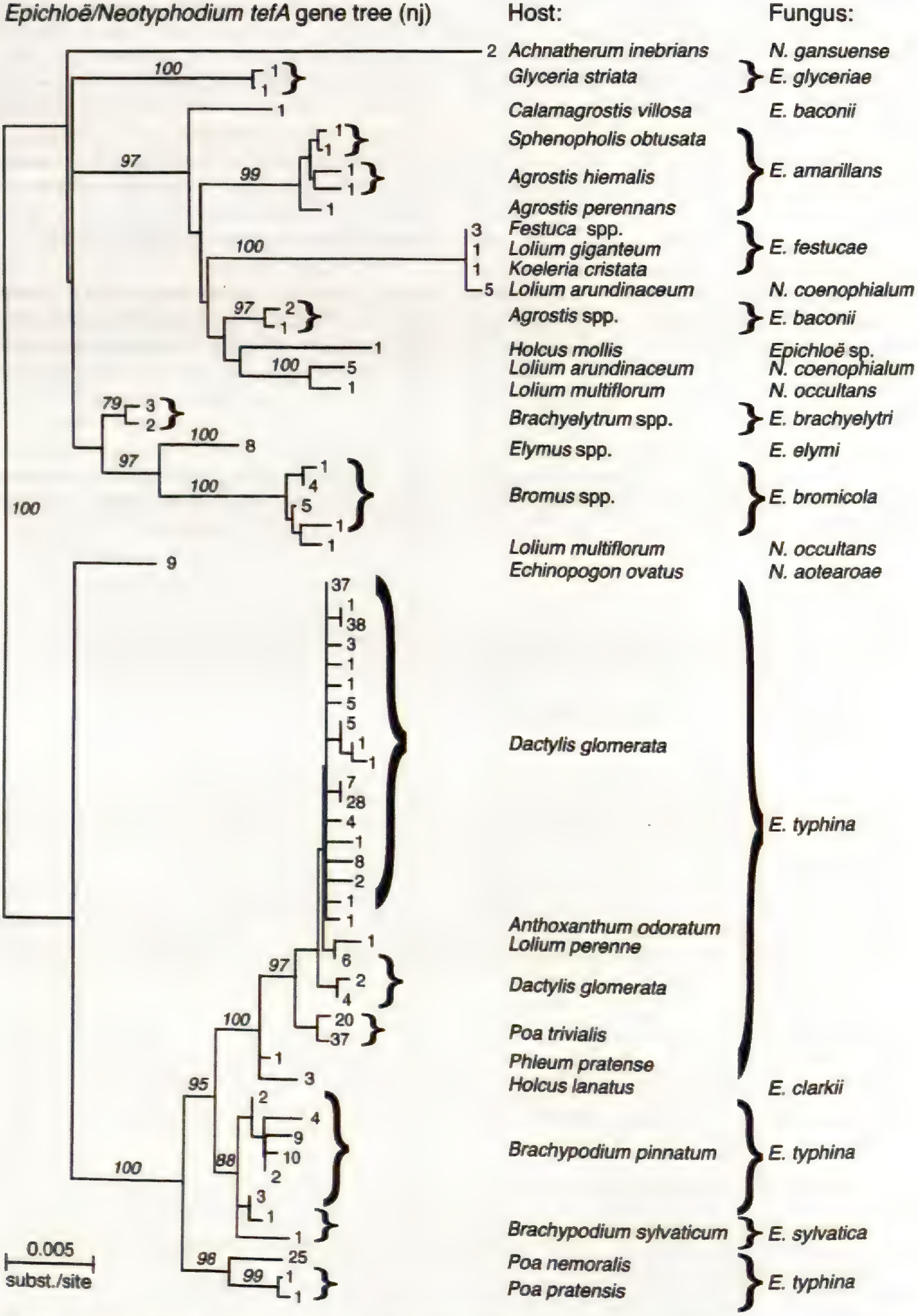

Figure 3. Phylogenetic relationships of *Epichloë* and *Neotyphodium* isolates from various host species based on maximum likelihood analysis (PhyML) of aligned *tefA* intron sequences. The number on each terminal of the tree indicates the number of isolates identified with the same allele sequence (haplotype). *Neotyphodium coenophialum* and *N. occultans* are each represented by two separate terminals, designating the two *tefA* alleles possessed by each of these interspecific hybrids. Most *Epichloë* species as currently circumscribed constitute monophyletic clades in this gene tree. Exceptions are *E. baconii*, which

host. Published studies (Chung et al., 1997) and personal observations of the author suggest that all of these factors may be involved, but that none of them constitutes an absolute isolation mechanism.

Epichloë stromata are hermaphroditic, expressing both spermatia and ascogenous (female) hyphae (White et al., 1991). The sexual cycle has not been reproduced in culture, but conidia produced in culture can serve as spermatia when transferred to a stroma of opposite mating type in the same interfertility group (Schardl & Tsai, 1992). For this reason, many isolates that do not produce stromata can be checked for mating compatibility with stroma-forming strains. For example, *E. bromicola* is known from several *Bromus* L. species and produces stromata on *B. erectus* Huds., but not on *B. benekenii* or *B. ramosus* Huds. Isolates from the latter two hosts were cultured and spermatia were placed on *E. bromicola* stromata (on *B. erectus*), with which they proved to be interfertile (Brem & Leuchtmann, 2003). This finding extended the known host range of *E. bromicola* and demonstrated that an *Epichloë* species may fruit on only some of its host species.

Several other epichloae also appear to have arisen from *Epichloë* species infecting new host species (host jumps), losing their ability to fruit, and becoming strictly seed transmitted (Fig. 4). These are the nonhybrid *Neotyphodium* species (the hybrids are discussed later). Some arose by jumps between species in the same grass genus, as in the aforementioned case of *E. bromicola*. Other apparent jumps are broader, between host genera or tribes. In three instances of jumps between host tribes, the fungus has retained the ability to mate with its ancestral *Epichloë* species if conidia from culture are used as spermatia. Thus, an isolate from *Hordelymus europaeus* (L.) Harz (tribe Triticeae) is compatible with *E. bromicola* on *Bromus erectus* (Bromeae), an isolate from *Elymus virginicus* L. (Triticeae) is compatible with *Epichloë amarillans* on *Agrostis perennans* (Walter) Tuck. (Poeae), and an isolate from *B. kalmii* A. Gray (Bromeae) is compatible with *Epichloë elymi* on *Elymus virginicus* (Triticeae) (Moon et al., 2004). These results establish clear relationships to sexual ancestors for epichloae that lack a sexual state in nature. In contrast, other epichloae arising from host jumps seem to have lost male fertility as well as fruiting ability.

The overall pattern emerges that each *Epichloë* species forms stromata mainly on hosts of a single species, genus, or group of related genera in a tribe, and that a strain that has colonized a more distantly related host does not fruit on that host. So far, this pattern seems to apply to almost every known *Epichloë* species and host-specific population of *E. typhina*, the sole exception being *E. festucae*, which infects and fruits on *Festuca* and *Lolium* species as well as the more distantly related *Koeleria pyramidata*. The pattern suggests that most *Epichloë* species can only fruit on a host with which it has coevolved.

I propose a trapped-symbiont hypothesis that, when an *Epichloë* species infects a host with which it has not coevolved, it may not express stromata in the new host and thereby becomes trapped in vertical transmission mode. This particular hypothesis has not been sufficiently tested because it is typically difficult to move an *Epichloë* or *Neotyphodium* isolate from one grass host into a host that does not naturally harbor the same species (Christensen, 1995). There is one reported case in which *Epichloë elymi* from *Elymus virginicus* was successfully moved to *Brachyelytrum erectum* (Schreb.) P. Beauv. and then apparently produced stromata on the artificially infected *B. erectum* plants (Leuchtmann & Clay, 1993). However, failure to reproduce this result (Schardl, unpublished) leaves some doubt about the identity of the observed stroma-forming plants. Another study that bears relevance to the trapped endophyte hypothesis is that of Brem and Leuchtmann (2003), who observed that natural associations of *Epichloë bromicola* with *Bromus erectus* produced stromata, and those with *B. benekenii* or *B. ramosus* did not; yet when isolates of *E. bromicola* from *B. erectus* were introduced into *B. benekenii* or *B. ramosus*, the fungus produced stromata in the new associations. This result is unsupportive of my trapped-endophyte hypothesis, with the caveats that the stromata on *B. benekenii* and *B. ramosus* caused atypical development of the host tillers (suppressing stem elongation), and that the new associations were unstable. Brem and Leuchtmann (2003) suggest that such instability provided selection favoring the strict vertical transmission that characterizes natural *E. bromicola* symbioses with *B. benekenii* and *B. ramosus*. Therefore, although it is conceivable that some host jumps immediately trap the symbiont and render it unable to fruit, others may

---

←
appears to be polyphyletic, and *E. typhina*, which appears to be paraphyletic to *E. clarkii* and *E. sylvatica*. In every species except *E. festucae*, isolates from each host genus have haplotypes not found in isolates from other host genera. Thus, a survey of 273 isolates of *E. typhina* from four sympatric host species indicated that no haplotypes were shared between isolates from different host species.

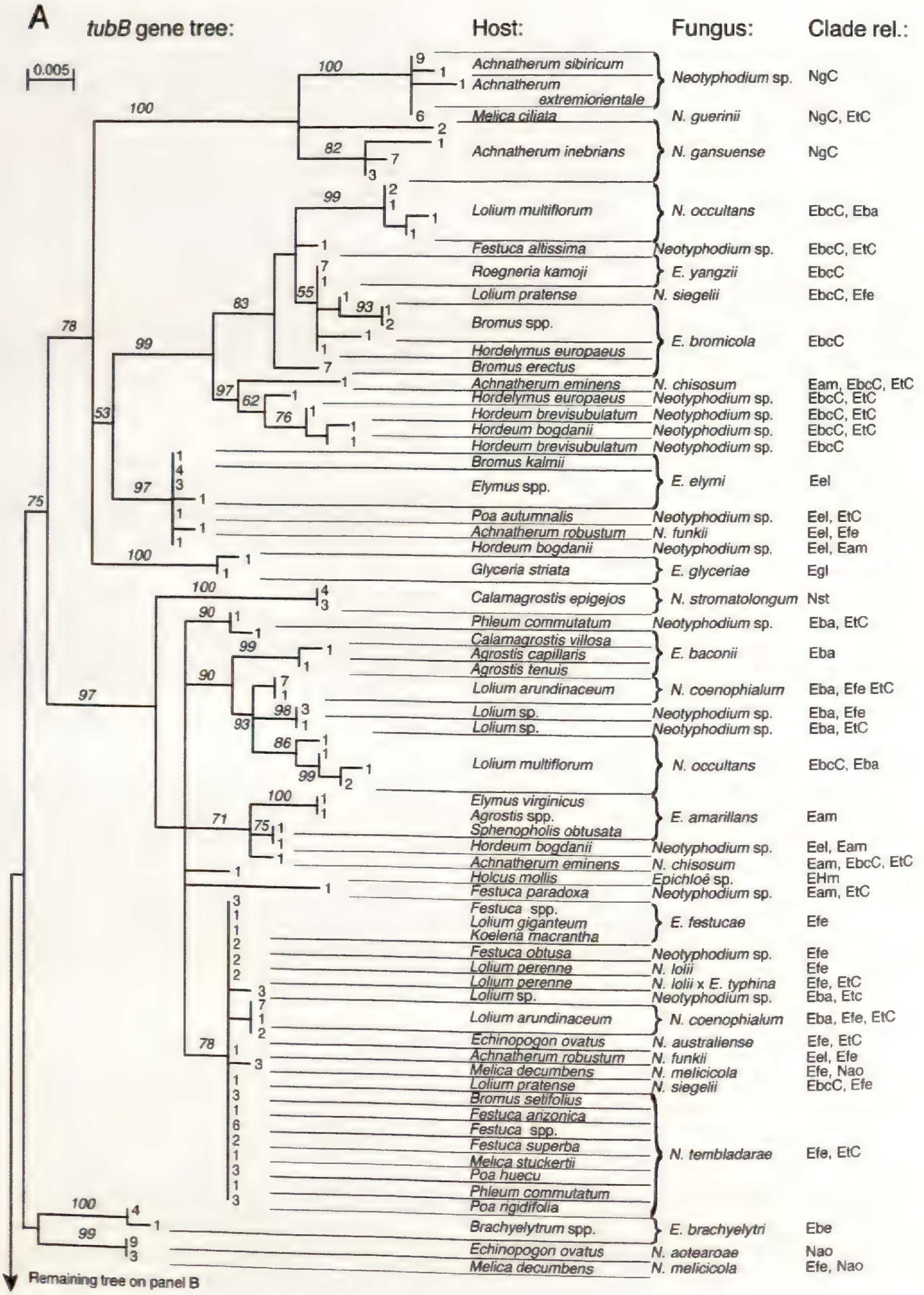
A
tubB gene tree:
Host:
Fungus:
Clade rel.:
0.005
Achnatherum sibiricum
Achnatherum extremiorientale
Neotyphodium sp. NgC
Melica ciliata N. guerinii NgC, EtC
Achnatherum inebrians N. gansuense NgC
Lolium multiflorum N. occultans EbcC, Eba
Festuca altissima Neotyphodium sp. EbcC, EtC
Roegneria kamoji E. yangzii EbcC
Lolium pratense N. siegelii EbcC, Efe
Bromus spp.
Hordelymus europaeus
Bromus erectus
E. bromicola EbcC
Achnatherum eminens N. chisosum Eam, EbcC, EtC
Hordelymus europaeus Neotyphodium sp. EbcC, EtC
Hordeum brevisubulatum Neotyphodium sp. EbcC, EtC
Hordeum bogdanii Neotyphodium sp. EbcC, EtC
Hordeum brevisubulatum Neotyphodium sp. EbcC
Bromus kalmii
Elymus spp.
E. elymi Eel
Poa autumnalis Neotyphodium sp. Eel, EtC
Achnatherum robustum N. funkii Eel, Efe
Hordeum bogdanii Neotyphodium sp. Eel, Eam
Glyceria striata E. glyceriae Egl
Calamagrostis epigejos N. stromatolongum Nst
Phleum commutatum Neotyphodium sp. Eba, EtC
Calamagrostis villosa
Agrostis capillaris
Agrostis tenuis
E. baconii Eba
Lolium arundinaceum N. coenophialum Eba, Efe EtC
Lolium sp. Neotyphodium sp. Eba, Efe
Lolium sp. Neotyphodium sp. Eba, EtC
Lolium multiflorum N. occultans EbcC, Eba
Elymus virginicus
Agrostis spp.
Sphenopholis obtusata
E. amarillans Eam
Hordeum bogdanii Neotyphodium sp. Eel, Eam
Achnatherum eminens N. chisosum Eam, EbcC, EtC
Holcus mollis Epichloë sp. EHm
Festuca paradoxa Neotyphodium sp. Eam, EtC
Festuca spp.
Lolium giganteum
Koeleria macrantha
E. festucae Efe
Festuca obtusa Neotyphodium sp. Efe
Lolium perenne N. lolii Efe
Lolium perenne N. lolii x E. typhina Efe, EtC
Lolium sp. Neotyphodium sp. Eba, Etc
Lolium arundinaceum N. coenophialum Eba, Efe, EtC
Echinopogon ovatus N. australiense Efe, EtC
Achnatherum robustum N. funkii Eel, Efe
Melica decumbens N. melicicola Efe, Nao
Lolium pratense N. siegelii EbcC, Efe
Bromus setifolius
Festuca arizonica
Festuca spp.
Festuca superba
Melica stuckertii
Poa huecu
Phleum commutatum
Poa rigidifolia
N. tembladarae Efe, EtC
Brachyelytrum spp. E. brachyelytri Ebe
Echinopogon ovatus N. aotearoae Nao
Melica decumbens N. melicicola Efe, Nao
Remaining tree on panel B

Figure 4. Phylogenetic relationships of asexual epichloae (*Neotyphodium* spp.) with sexual epichloae (*Epichloë* spp.) based on maximum likelihood analysis (PhyML) of aligned *tubB* intron sequences. —A. Sequences outside the *E. typhina* complex (*EtC*) clade. —B. Sequences in the *EtC* clade. Listed are host species, fungal species (if described), and the clade relationships of the fungal *tubB* gene sequences.

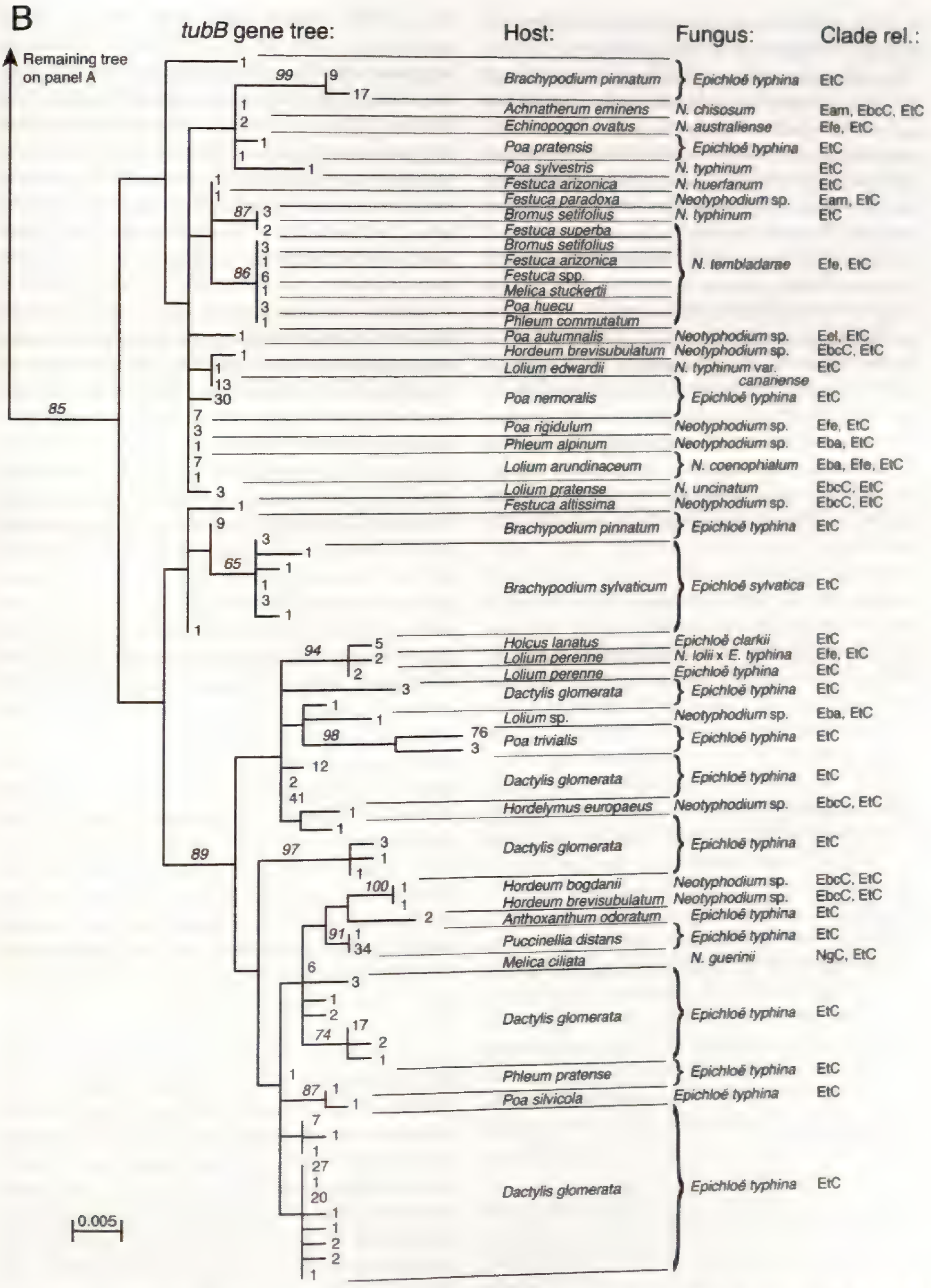

Figure 4 (Continued). The number on each terminal leaf of the tree indicates the number of isolates identified with the same allele sequence. Numbers on branches are bootstrap support values. All haploids have only one detectable *tubB* allele, whereas those with multiple alleles are inferred to be interspecific hybrids with ancestors belonging to the clades indicated. Major clades are abbreviated as in Table 1. The tree was inferred by PhyML with a general time-reversible model of nucleotide substitutions, estimated proportion of invariant sites (= 0.343), and estimated gamma shape parameter (= 0.891) assuming four rate categories.

gradually evolve to that stage under selection pressure for greater stability and mutual benefit.

Up to this point, I have implied that loss of fruiting (either by mutation and selection, or by host effects) confines the epichloid fungus to vertical transmission mode, but such an assertion needs critical assessment. Ascospores form in the stromata, and studies under natural or near-natural conditions have indicated that ascospores mediate horizontal transmission (Chung & Schardl, 1997a; Brem & Leuchtmann, 1999; Leyronas & Raynal, 2008). Conidia appear less likely to be involved in horizontal transmission, and in any case are mainly associated with the newly emerged stromata. Conidia produced on asymptomatic plant surfaces might conceivably mediate horizontal transmission (Tadych et al., 2007), but epiphyllous conidia are very sparse compared with the stroma. With these considerations, it seems apparent that without stroma production an epichloid lineage is highly, but perhaps not entirely, dependent on vertical transmission for its persistence.

With the exception of asexual epichloae (*Neotyphodium* species), stroma production (either borne on the plant or arising from a sclerotium) is a feature shared by all plant-associated Clavicipitaceae. Therefore, it is likely that the stroma is required for these fungi to complete their life cycle and transmit to new host individuals. What sets the *Epichloë* species apart is their highly efficient vertical transmission, which obviates any requirement for fruiting and the sexual cycle, at least on short evolutionary time scales (Selosse & Schardl, 2007). *Balansia hypoxylon* (Peck) G. F. Atk. [≡ *Atkinsonella hypoxylon* (Peck) Diehl] also transmits vertically via cleistogamous seeds of infected grass species within *Danthonia* DC., but a population-level genetic study indicated that ascospore-mediated horizontal transmission is much more important for *B. hypoxylon* (Kover et al., 1997). Considering that vertical transmission is very common in *Epichloë*, that many *Epichloë* and *Neotyphodium* species provide fitness enhancements and protection to their hosts, and that hosts for epichloae are broadly distributed in subfamily Poöideae, the evolutionary origins of the Poöideae-epichloae symbioses is fascinating to consider.

EVOLUTION AND COPHYLOGENY OF POÖIDEAE-EPICHLOAE SYMBIOSES

Tribes of grass subfamily Poöideae known to include hosts of epichloae are Brachyelytreae, Brachypodieae, Bromeae, Diarrheneae, Meliceae, Nardieae, Poeae, Stipeae, and Triticeae. The few tribes missing from this list are certainly understudied, so it would not be surprising to find that they also include hosts of epichloae. Known hosts represent the entire phylogenetic range of Poöideae, from *Brachyelytrum erectum* to *Poa pratensis* L., but remarkably there are none documented outside of the Poöideae. Rather, members of other grass subfamilies (and more rarely, other monocot and even dicot families) host other Clavicipitaceae. This pattern of host and symbiont relationships suggests that the genus *Epichloë* evolved at approximately the same time as the Poöideae, perhaps as cospeciation events.

Some of the relationships within the epichloae suggest that cospeciation, or a more diffuse process of cophylogeny, has been common, but not universal, throughout the evolution of the Poöideae and *Epichloë* species (Schardl et al., 1997). The pattern seems to be obscured somewhat by the large number of interspecific hybrids as well as the apparently broad host-range *E. typhina* complex. If these are removed from consideration, then the major clades of the remaining epichloae reflect major clades of the grasses (Figs. 3, 4). The speciose tribe Poeae hosts several related species, *E. baconii*, *E. amarillans*, and *E. festucae*, as well as *Neotyphodium stromatolongum*, and an undescribed species associated with *Holcus mollis* L. Similarly, *E. elymi*, *E. yangzii*, and *E. bromicola* are symbionts of grasses in the sister tribes Triticeae and Bromeae. Deep branches in the *Epichloë-Neotyphodium* gene trees correspond with deep divergences of host tribes Brachyelytreae, Stipeae, and Meliceae (Schardl et al., 2008). The topologies of the *tefA* and *tubB* gene trees are not identical (Figs. 3, 4), and there remains ambiguity in the topology of the Poöideae tree, making any precise topological comparison impossible. An alternative method to assess cophylogenetic relationships is by comparing pairwise distance matrices, but because this approach heavily weights deeper nodes (older divergences) relative to shallower nodes (recent divergences), we modified the method to correct for this disparity (Schardl et al., 2008). The method takes account of topology, but tests for statistically significant correspondence of ages of corresponding nodes in the host and symbiont gene trees. Applying this method to fungal *tefA* and *tubB* sequences and to intergenic and intron sequences of the plant chloroplast genome indicated significant support to reject the null hypothesis that the phylogenies were unrelated. This evidence for cophylogeny of epichloae and host grasses suggests that the genus *Epichloë* originated approximately with the origin of the Poöideae, 30–40 million years ago. If so, the common ancestors of host and symbiont probably interacted much like the current-day *Brachyelytrum–Epichloë brachyelytri*, *Festuca–Epichloë festucae*, and most other Poöideae-*Epichloë* symbioses with mixed horizontal and vertical symbiont transmission.

Poöideae–*Epichloë* symbioses are unique among the interactions of Poaceae with Clavicipitaceae in that both the plant and the symbiont are fully capable of both asexual and sexual reproduction, and the symbiont is efficiently transmitted in the host seed progeny. Although most other Poaceae-Clavicipitaceae symbioses lack vertical transmissibility, the *Danthonia–Balansia hypoxylon* symbioses most closely resemble the Poöideae-*Epichloë* symbioses in this respect (Clay, 1994; Kover & Clay, 1998). In *Danthonia* species, *B. hypoxylon* transmits vertically in cleistogamous (self-fertilized) seeds, but almost always chokes the chasmogamous (open-pollinated) inflorescences. Sometimes, particularly when *B. hypoxylon* strains are moved between different *Danthonia* species, chasmogamous inflorescences are incompletely choked and produce open-pollinated seeds, some of which transmit the fungus. Apparently, there is a trade-off between virulence and vertical transmissibility to chasmogamous, as well as cleistogamous, seed progeny in the *Danthonia–Balansia hypoxylon* system (Kover & Clay, 1998). In contrast, the Poöideae-*Epichloë* system exhibits no such trade-off. In those cases where an *Epichloë* strain infrequently chokes its host, vertical transmission to seed progeny is highly efficient, whereas some, more virulent *Epichloë* strains are apparently incapable of vertical transmission in their hosts (Chung & Schardl, 1997a). Thus, the key advance in the evolution of the Poöideae-*Epichloë* system may have been to break the link between virulence and transmissibility. With that link broken, hosts can reward relatively benign symbionts by providing highly efficient vertical dissemination and can deny this benefit to highly virulent strains.

ASEXUAL EPICHLOAE, THE *NEOTYPHODIUM* SPECIES

Given that Poöideae vertically transmit the epichloae at very high efficiency and that the symbionts have the potential to be highly beneficial by, for example, combating herbivores, the most favorable situation for the host may be a symbiosis without choke. Such a symbiosis relegates the symbiont to strictly clonal reproduction; that is, the fungus is asexual. Due to Article 59 of the *International Code of Botanical Nomenclature* (McNeill et al., 2006), such asexual fungi are artificially placed in a separate genus from their sexual relatives, so in a formal sense the asexual epichloae are classified as *Neotyphodium* species, although they are clear phylogenetic congeners of the sexual *Epichloë* species (Kuldau et al., 1997; Moon et al., 2004). Although the asexual *Neotyphodium* species fail to produce stromata, some can be experimentally mated to compatible *Epichloë* strains because conidia produced in culture can serve as spermatia. Most *Neotyphodium* species, however, seem truly to be asexual. Such species disseminate mainly—indeed, perhaps exclusively—by vertical transmission, which is accomplished when they invade the ovule and eventually the developing embryo without any negative effects on the host tissues (Freeman, 1904). When the seed germinates, the fungus continues to colonize the shoot apical meristems. As plant cells emerge from the meristematic zone into the expansion zone, the fungal symbiont keeps pace apparently by intercalary growth of its hyphal strands (Christensen et al., 2008). Colonization of new tillers tends to be highly efficient, and once floral primordia are initiated they are also colonized, allowing for vertical transmission in the next host generation.

Considering that this same process of vertical transmission is exemplified by many *Epichloë* species–host species interactions, the simple scenario for evolution of *Neotyphodium* species would be that loss of the sexual state, for example by loss of key genes for stroma production, would generate an asexual fungus. This fungus would remain fit and persist in nature by virtue of its very efficient vertical transmission, and because this would remain more likely to enhance than degrade host fitness, selection would favor host compatibility. This simple scenario, however, appears not to apply to most of the asexual epichloae, which have more complex evolutionary origins; most have the genetic footprints of interspecific hybrid origins.

A major difference between the genomes of the sexual and asexual epichloae is that the sexual species are generally haploid, whereas most (but by no means all) of the asexual species are heteroploid; that is, they possess more than a single complement of chromosomes. Evidence for this includes the detection of multiple alleles in asexual isolates for genes that are singly present in each sexual isolate (Leuchtmann & Clay, 1989; Moon et al., 2004). Further evidence is the difference in genome sizes. Quantitative Southern-blot analysis and electrophoretic karyotyping indicate that *Neotyphodium coenophialum* and a *N. lolii* × *Epichloë typhina* hybrid (an undescribed taxon identified as LpTG-2) have approximately twice the genomic DNA content as isolates of the sexual species *E. festucae*, *E. typhina*, and the nonhybrid asexual species *N. lolii* (Kuldau et al., 1999). The *E. festucae* genome has been sequenced and subjected to optical mapping (Schardl, unpublished), and the results indicate a genome size of 27–30 megabases (Mb), almost precisely the size estimated by Kuldau et al.

(1999). The estimated 57-Mb genome size of *N. coenophialum* is likely, therefore, to be similarly accurate.

Most *Neotyphodium* species have multiple (two or sometimes three) alleles of genes for which only a single allele is detectable in sexual *Epichloë* species. Phylogenetic analyses of *tubB*, *tefA*, and *actG* sequences have been conducted to distinguish the possibilities that the multiple alleles in heteroploid epichloae arose by gene duplications, or alternatively were contributed by different ancestors (Schardl et al., 1994; Moon et al., 2004; Gentile et al., 2005; Moon et al., 2007; Iannone et al., 2009; Yan et al., 2009). In each case, the different alleles in an isolate showed relationships to those of different sexual species (Fig. 4). Each *Epichloë* species occupies a distinct clade in the phylogeny, although there are some cases of paraphyly. The relationships among multiple alleles in each asexual species are much more divergent than the relationships among alleles within any population of sexual species. Furthermore, most of the alleles found in the asexual epichloae are similar or identical to alleles in *Epichloë* species, making it possible to assign ancestry of the hybrid endophytes. In Figure 4, each hybrid has ancestors from the two or three distinct species or clades indicated. These ancestors are apparent from the relationships of their two or three *tubB* alleles, except in the case of *N. uncinatum*, which has only one detectable *tubB* allele from the *E. typhina* clade. However, the single *tefA* allele of *N. uncinatum* is related to that of *E. bromicola*, and this fungus has two *actG* alleles related to those of *E. typhina* and *E. bromicola* (Craven et al., 2001a).

The hybridization events giving rise to asexual epichloae were most likely parasexual (somatic) rather than sexual. Extensive attempts to generate sexual hybrids almost all failed, with the exception of an *Epichloë festucae* × *E. baconii* mating (Leuchtmann & Schardl, 1998). The resulting progeny was haploid, with parental alleles that had segregated in Mendelian fashion. Thus, this sexual hybridization failed to generate progeny with the typical heteroploid constitution. Also, two asexual epichloae, *Neotyphodium coenophialum* (Tsai et al., 1994) and *N. chisosum* (Moon et al., 2007), have three ancestral species each. Considering that sexual isolates are consistently haploid, a sexual origin is difficult to imagine for these three-ancestor hybrids. Nevertheless, the sexual state was likely involved in hybridization events because a horizontal transmission event was required to move an ancestral *Epichloë* strain into a host in which the other ancestor resided (Fig. 5).

Initially, evidence of interspecific hybrid origins of *Neotyphodium coenophialum* and other *Neotyphodium* species (Schardl et al., 1994; Tsai et al., 1994) was surprising in light of a widespread belief that all filamentous ascomycetes possess systems of vegetative incompatibility, which require identical alleles at several vegetative incompatibility (*vic*) loci in order for the fungi to maintain heterokaryons after fusing hyphae during plasmogamy (Glass et al., 2000). Vegetatively incompatible strains produce a barrage reaction upon plasmogamy, killing the heterokaryotic hyphae. Somatic origin of hybrid *Neotyphodium* species required plasmogamy without a barrage response, and the chance that two different species would have identical alleles at several *vic* loci seemed remote. An investigation of vegetative compatibility between *Epichloë* species indicated that this classical vegetative compatibility system does not operate in the genus (Chung & Schardl, 1997b). Therefore, heterokaryons could form and represent the first step in hybridization. However, subsequent fusion of nuclei (karyogamy) was also required to give the heteroploid nuclear genotypes that typify hybrid epichloae, and no such events were detected. Furthermore, experimental coinfections of host grasses with two different epichloae have not yet yielded demonstrable hybrids. Instead, the typical observation is that the fungal strains segregate from one another in different tillers of the plant (Wille et al., 1999; Christensen et al., 2000a).

The difficulty in producing interspecific hybrids experimentally begs the question, why are such hybrid epichloae so common? One possibility is that they occur in circumstances that are atypical of the experimental conditions. For example, perhaps they occur only in the developing seed when it becomes superinfected by two epichloae, one from the maternal parent, and the other from an ascospore that infected the floret. It may be that some unknown peculiarity of fungal development in the seed makes plasmogamy more likely there than in other plant tissues. Alternatively, the hybridizations may be extremely rare, but hybrids are strongly selected. It is worth considering that as host plants evolve, their symbionts may also need to evolve in response, or otherwise be lost. The evolutionary potential of a clonal fungus is far less than that of a sexual fungus, so the parasexual process of hybridization may be necessary to facilitate sufficiently rapid evolution of the otherwise clonal fungus. It is also noteworthy that many of the associated grasses are polyploids that arose by interspecific hybridization, though there is no clear association of polyploid hosts and hybrid epichloae. Finally, the parasexual cycle might counteract the progression of deleterious mutations, which is expected on the basis of Muller's ratchet (Moon et al., 2004; Selosse & Schardl, 2007).

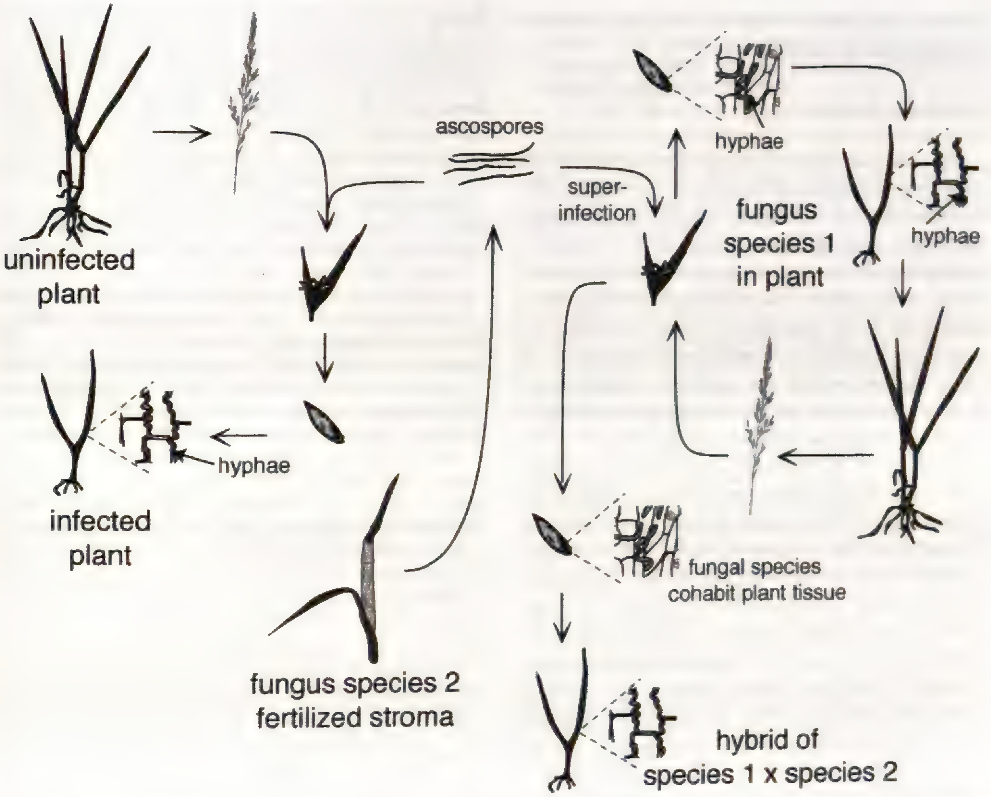
uninfected plant
ascospores
super-infection
hyphae
fungus species 1 in plant
hyphae
hyphae
infected plant
fungal species cohabit plant tissue
fungus species 2 fertilized stroma
hybrid of species 1 x species 2

Figure 5. Proposed scenario for the origin of hybrid epichloae. Ascospores produced by an *Epichloë* species may infect seeds produced on uninfected plants (left) or superinfect seeds produced on plants with a resident epichloë symbiont (right). In this scenario, hybridization is assumed to occur early in the superinfection before the symbionts can segregate from one another in different host tillers.

There are three curious but unexplained patterns in relationships of epichloae and their hosts. One is that host species may possess either vertically transmissible epichloae or the more pathogenic strains that are not vertically transmitted, but rarely both. Hence, the *Epichloë* strains infecting *Anthoxanthum odoratum* L., *Brachypodium pinnatum*, *Bromus erectus*, *Dactylis glomerata*, *Glyceria striata*, *Holcus lanatus* L., *Phleum pratense* L., *Poa sylvicola* Guss., and *Poa trivialis* almost completely prevent seed production on infected plants; their vertical transmission has not been observed; and no vertically transmitted *Epichloë* or *Neotyphodium* species have been observed in any of these hosts (aside from *Acremonium chilense*, which may be vertically transmissible in *D. glomerata* [Morgan-Jones et al., 1990] but is not closely related to *Epichloë* species). The exception is *Lolium perenne*, in which *N. lolii* is a common, vertically transmitted symbiont, whereas *E. typhina* is much rarer and not vertically seed transmissible in this host (Leuchtmann & Schardl, 1998). Second, some host grass species have more than one species of vertically transmissible epichloae, but at most one of these is nonhybrid and the others are interspecific hybrids. So, for example, *Festuca arizonica* Vasey is a host for the hybrid *N. tembladerae* Cabral & J. F. White and the nonhybrid *N. huerfanum* (J. F. White, G. T. Cole & Morgan-Jones) A. E. Glenn, C. W. Bacon & R. T. Hanlin (Moon et al., 2004; Sullivan & Faeth, 2008) (Fig. 4). Finally, the two or three identifiable ancestors of each hybrid *Neotyphodium* species are from different, well-separated phylogenetic clades (Fig. 4). As more isolates are surveyed, more exceptions to these patterns may be found, but the fact that they are evident among the many epichloae studied to date suggests that these patterns reflect biological phenomena. Although these phenomena have yet to be fully elucidated, it seems likely that they must reflect aspects of selection on the systems. For example, I speculate that the highly pathogenic *Epichloë* species select for a form of resistance in their hosts such that the plants cannot support vertical transmission, whereas less pathogenic epichloae provide less selection against, or even selection favoring, vertical transmission.

CONCLUDING REMARKS

The plant-associated Clavicipitaceae present a rich variety of experimental material to investigate the mechanisms and evolution across an ecological continuum that includes antagonistic symbioses, mutualistic symbioses, and symbioses that defy simple classification because they vary in relative benefits or detriments to the host depending on developmental stage and ecological circumstance. Characteristics of these systems include the capability of host plants, mainly grasses, and certain clavicipitaceous fungi to maintain long-term systemic associations, and the capabilities of the clavicipitaceous fungi to produce toxins that deter or kill grazers. These characteristics appear key to the evolution of mutualism commonly, but not universally, observed in Poöideae-epichloae symbioses. In the extreme cases of grass symbioses with asexual epichloae (*Neotyphodium* species), vertical transmission is highly efficient and is the main or sole means of dissemination. Significant protective effects on the host grasses are consistent with the prediction that selection on vertically transmitted symbionts favors host benefits (Ewald, 1987). Such protective mutualisms have evolved more than once in the Clavicipitaceae, which also include vertically transmitted, ergot alkaloid–producing symbionts of *Ipomoea* and *Turbina* species (Convolvulaceae).

Many other Clavicipitaceae share characteristics of the epichloae in that they infect plants systemically but cause no damage to most infected tissues, and they produce antiherbivore alkaloids. But, in contrast to the other Clavicipitaceae, including the vertically transmissible *Balansia hypoxylon*, most epichloae as well as the symbionts of *Ipomoea* and *Turbina* species can transmit vertically without disrupting host development. Thus, what sets apart the clavicipitaceous symbionts of Poöideae and Convolvulaceae is that the link between transmissibility and virulence has been removed in the course of their coevolution. Evidence that evolution of these mutualistic symbionts coincided with the origin of their host grasses has prompted speculation that the symbiotic epichloae may have helped in the transition of pooid grasses into open habitats (Schardl et al., 2008).

Literature Cited

Bacetty, A., M. Snook, A. Glenn, J. Noe, P. Nagabhyru & C. Bacon. 2009. Chemotaxis disruption in *Pratylenchus scribneri* by tall fescue root extracts and alkaloids. J. Chem. Ecol. 35: 844–850.

Barkworth, M. E., K. M. Capels, S. Long, L. K. Anderton & M. B. Piep (editors). 2007. Flora of North America North of Mexico, Vol 24. Magnoliophyta: Commelinidae (in part): Poaceae, Pt. 1. Oxford University Press, New York.

Bischoff, J. F. & J. F. White Jr. 2003. The plant-infecting clavicipitaleans. Pp. 125–150 *in* J. F. White Jr., C. W. Bacon, N. L. Hywel-Jones & J. W. Spatafora (editors), Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts. Marcel-Dekker Inc., New York.

Brem, D. & A. Leuchtmann. 1999. High prevalence of horizontal transmission of the fungal endophyte *Epichloë sylvatica*. Bull. Geobot. Inst. ETH 65: 3–12.

——— & ———. 2001. *Epichloë* grass endophytes increase herbivore resistance in the woodland grass *Brachypodium sylvaticum*. Oecologia 126: 522–530.

——— & ———. 2003. Molecular evidence for host-adapted races of the fungal endophyte *Epichloë bromicola* after presumed host shifts. Evolution 57: 37–51.

Bultman, T. L., J. F. White Jr., T. I. Bowdish & A. M. Welch. 1998. A new kind of mutualism between fungi and insects. Mycol. Res. 102: 235–238.

Bush, L. P., H. H. Wilkinson & C. L. Schardl. 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. Pl. Physiol. 114: 1–7.

Cheplick, G. P. & K. Clay. 1988. Acquired chemical defences in grasses: The role of fungal endophytes. Oikos 52: 309–318.

Christensen, M. J. 1995. Variation in the ability of *Acremonium* endophytes of *Lolium perenne*, *Festuca arundinacea* and *F. pratensis* to form compatible associations in the three grasses. Mycol. Res. 99: 466–470.

———, A. Leuchtmann, D. D. Rowan & B. A. Tapper. 1993. Taxonomy of *Acremonium* endophytes of tall fescue (*Festuca arundinacea*), meadow fescue (*F. pratensis*), and perennial rye-grass (*Lolium perenne*). Mycol. Res. 97: 1083–1092.

———, W. R. Simpson & T. Al Samarrai. 2000a. Infection of tall fescue and perennial ryegrass plants by combinations of different *Neotyphodium* endophytes. Mycol. Res. 104: 974–978.

———, K. Takahashi, K. Kohnon, T. Tsukiboshi & M. Kobayashi. 2000b. Occurrence of an *Ephelis* fungus on Ishigaki Island and observations on its epiphytic association with host grasses. JIRCAS J. 8: 49–59.

———, R. J. Bennett, H. A. Ansari, H. Koga, R. D. Johnson, G. T. Bryan, W. R. Simpson, J. P. Koolaard, E. M. Nickless & C. R. Voisey. 2008. *Epichloë* endophytes grow by intercalary hyphal extension in elongating grass leaves. Fung. Genet. Biol. 45: 84–93.

Chung, K.-R. & C. L. Schardl. 1997a. Sexual cycle and horizontal transmission of the grass symbiont, *Epichloë typhina*. Mycol. Res. 101: 295–301.

——— & ———. 1997b. Vegetative compatibility between and within *Epichloë* species. Mycologia 89: 558–565, 976.

———, W. Hollin, M. R. Siegel & C. L. Schardl. 1997. Genetics of host specificity in *Epichloë typhina*. Phytopathology 87: 599–605.

Clay, K. 1994. Hereditary symbiosis in the grass genus *Danthonia*. New Phytol. 126: 223–231.

———, G. P. Cheplick & S. Marks. 1989. Impact of the fungus *Balansia henningsiana* on *Panicum agrostoides*: Frequency of infection, plant growth and reproduction and resistance to pests. Oecologia 80: 374–380.

———, S. Marks & G. P. Cheplick. 1993. Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. Ecology 74: 1767–1777.

———, J. Holah & J. A. Rudgers. 2005. Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. Proc. Natl. Acad. Sci. U.S.A. 102: 12465–12470.

Craven, K. D., J. D. Blankenship, A. Leuchtmann, K. Hignight & C. L. Schardl. 2001a. Hybrid fungal endophytes symbiotic with the grass *Lolium pratense*. Sydowia 53: 44–73.

———, P. T. W. Hsiau, A. Leuchtmann, W. Hollin & C. L. Schardl. 2001b. Multigene phylogeny of *Epichloë* species, fungal symbionts of grasses. Ann. Missouri Bot. Gard. 88: 14–34.

Diehl, W. W. 1950. *Balansia* and the Balansiae in America. United States Department of Agriculture, Washington, D.C.

Ellis, J. B. & B. M. Everhart. 1886. Synopsis of the North American Hypocreaceae, with descriptions of the species (continued). J. Mycol. 2: 49–51.

Ewald, P. W. 1987. Transmission modes and evolution of the parasitism-mutualism continuum. Ann. New York Acad. Sci. 503: 295–306.

Faeth, S. H. 2002. Are endophytic fungi defensive plant mutualists? Oikos 98: 25–36.

Freeman, E. M. 1904. The seed fungus of *Lolium temulentum* L., the darnel. Philos. Trans., Ser. B 196: 1–27.

Gentile, A., M. S. Rossi, D. Cabral, K. D. Craven & C. L. Schardl. 2005. Origin, divergence, and phylogeny of *Epichloë* endophytes of native Argentine grasses. Molec. Phylogen. Evol. 35: 196–208.

Glass, N. L., D. J. Jacobson & P. K. T. Shiu. 2000. The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. Annual Rev. Genet. 34: 165–186.

Glenn, A. E., C. W. Bacon, R. Price & R. T. Hanlin. 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. Mycologia 88: 369–383.

———, D. M. Rykard, C. W. Bacon & R. T. Hanlin. 1998. Molecular characterization of *Myriogenospora atramentosa* and its occurrence on some new hosts. Mycol. Res. 102: 483–490.

Gonthier, D. J., T. J. Sullivan, K. L. Brown, B. Wurtzel, R. Lawal, K. VandenOever, Z. Buchan & T. L. Bultman. 2008. Stroma-forming endophyte *Epichloë glyceriae* provides wound-inducible herbivore resistance to its grass host. Oikos 117: 629–633.

Hesse, U., W. Schoberlein, L. Wittenmayer, K. Forster, K. Warnstorff, W. Diepenbrock & W. Merbach. 2005. Influence of water supply and endophyte infection (*Neotyphodium* spp.) on vegetative and reproductive growth of two *Lolium perenne* L. genotypes. Eur. J. Agron. 22: 45–54.

Iannone, L. J., D. Cabral, C. L. Schardl & M. S. Rossi. 2009. Phylogenetic divergence, morphological and physiological differences distinguish a new *Neotyphodium* endophyte species in the grass *Bromus auleticus* from South America. Mycologia 101: 340–351.

Kellogg, E. A. 2001. Evolutionary history of the grasses. Pl. Physiol. 125: 1198–1205.

Knaus, H. G., O. B. McManus, S. H. Lee, W. A. Schmalhofer, M. Garcia-Calvo, L. Helms, M. Sanchez, K. Giangiacomo, J. P. Reuben, A. Smith III, G. J. Kaczorowski & M. L. Garcia. 1994. Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels. Biochemistry 33: 5819–5828.

Kover, P. X. & K. Clay. 1998. Trade-off between virulence and vertical transmission and the maintenance of a virulent plant pathogen. Amer. Naturalist 152: 165–175.

———, T. E. Dolan & K. Clay. 1997. Potential versus actual contribution of vertical transmission to pathogen fitness. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 264: 903–909.

Kuldau, G. A., J. S. Liu, J. F. White Jr., M. R. Siegel & C. L. Schardl. 1997. Molecular systematics of Clavicipitaceae supporting monophyly of genus *Epichloë* and form genus *Ephelis*. Mycologia 89: 431–441.

———, H.-F. Tsai & C. L. Schardl. 1999. Genome sizes of *Epichloë* species and anamorphic hybrids. Mycologia 91: 776–782.

Leuchtmann, A. & K. Clay. 1988. *Atkinsonella hypoxylon* and *Balansia cyperi*, epiphytic members of the Balansiae. Mycologia 80: 192–199.

——— & ———. 1989. Isozyme variation in the fungus *Atkinsonella hypoxylon* within and among populations of its host grasses. Canad. J. Bot. 67: 2600–2607.

——— & ———. 1993. Nonreciprocal compatibility between *Epichloë* and four host grasses. Mycologia 85: 157–163.

——— & C. L. Schardl. 1998. Mating compatibility and phylogenetic relationships among two new species of *Epichloë* and other congeneric European species. Mycol. Res. 102: 1169–1182.

———, ——— & M. R. Siegel. 1994. Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. Mycologia 86: 802–812.

———, D. Schmidt & L. P. Bush. 2000. Different levels of protective alkaloids in grasses with stroma-forming and seed-transmitted *Epichloë/Neotyphodium* endophytes. J. Chem. Ecol. 26: 1025–1036.

Leyronas, C. & G. Raynal. 2008. Role of fungal ascospores in the infection of orchardgrass (*Dactylis glomerata*) by *Epichloë typhina* agent of choke disease. J. Pl. Pathol. 90: 15–21.

Malinowski, D. P. & D. P. Belesky. 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. Crop Sci. 40: 923–940.

———, A. Leuchtmann, D. Schmidt & J. Nösberger. 1997. Growth and water status in meadow fescue (*Festuca pratensis*) is affected by *Neotyphodium* and *Phialophora* endophytes. Agron. J. 89: 673–678.

Markert, A., N. Steffan, K. Ploss, S. Hellwig, U. Steiner, C. Drewke, S.-M. Li, W. Boland & E. Leistner. 2008. Biosynthesis and accumulation of ergoline alkaloids in a mutualistic association between *Ipomoea asarifolia* (Convolvulaceae) and a clavicipitalean fungus. Pl. Physiol. 147: 296–305.

McKay, A. C. & K. M. Ophel. 1993. Toxigenic *Clavibacter/Anguina* associations infecting grass seedheads. Ann. Rev. Phytopathol. 31: 151–167.

McNeill, J., F. R. Barrie, H. M. Burdet, V. Demoulin, D. L. Hawksworth, K. Marhold, D. H. Nicolson, J. Prado, P. C. Silva, J. E. Skog, J. H. Wiersema & N. J. Turland (editors). 2006. International Code of Botanical Nomenclature (Vienna Code). Regnum Veg. 146.

Miles, C. O., G. A. Lane, M. E. Di Menna, I. Garthwaite, E. L. Piper, O. J. P. Ball, G. C. M. Latch, J. M. Allen, M. B. Hunt, L. P. Bush, F. K. Min, I. Fletcher & P. S. Harris. 1996. High levels of ergonovine and lysergic acid amide in toxic *Achnatherum inebrians* accompany infection by an *Acremonium*-like endophytic fungus. J. Agric. Food Chem. 44: 1285–1290.

Moon, C. D., K. D. Craven, A. Leuchtmann, S. L. Clement & C. L. Schardl. 2004. Prevalence of interspecific hybrids amongst asexual fungal endophytes of grasses. Molec. Ecol. 13: 1455–1467.

———, J.-J. Guillaumin, C. Ravel, C. Li, K. D. Craven & C. L. Schardl. 2007. New *Neotyphodium* endophyte species from the grass tribes Stipeae and Meliceae. Mycologia 99: 895–905.

Morgan-Jones, G. & J. F. White Jr. 1989. Concerning *Atkinsonella texensis*, a pathogen of the grass *Stipa leucotrichia*: Developmental morphology and mating system. Mycotaxon 35: 455–467.

———, ——— & E. L. Pointelli. 1990. Endophyte-host associations in forage grasses. 8. *Acremonium chilense*, an undescribed endophyte occuring in *Dactylis glomerata* in Chile. Mycotaxon 39: 441–454.

Omacini, M., E. J. Chaneton, C. M. Ghersa & C. B. Muller. 2001. Symbiotic fungal endophytes control insect host-parasite interaction webs. Nature 409: 78–81.

Panaccione, D. G., J. R. Cipoletti, A. B. Sedlock, K. P. Blemings, C. L. Schardl, C. Machado & G. E. Seidel. 2006a. Effects of ergot alkaloids on food preference and satiety in rabbits, as assessed with gene-knockout endophytes in perennial ryegrass (*Lolium perenne*). J. Agric. Food Chem. 54: 4582–4587.

———, J. B. Kotcon, C. L. Schardl, R. D. Johnson & J. B. Morton. 2006b. Ergot alkaloids are not essential for endophytic fungus-associated population suppression of the lesion nematode, *Pratylenchus scribneri*, on perennial ryegrass. Nematology 8: 583–590.

Parker, E. J. & D. B. Scott. 2004. Indole-diterpene biosynthesis in ascomycetous fungi. Pp. 405–426 *in* Z. An (editor), Handbook of Industrial Mycology. Marcel Dekker Inc., New York.

Petroski, R., R. G. Powell & K. Clay. 1992. Alkaloids of *Stipa robusta* (sleepygrass) infected with an *Acremonium* endophyte. Nat. Toxins 1: 84–88.

Potter, D. A., J. T. Stokes, C. T. Redmond, C. L. Schardl & D. G. Panaccione. 2008. Contribution of ergot alkaloids to suppression of a grass-feeding caterpillar assessed with gene-knockout endophytes in perennial ryegrass. Entomol. Exp. Appl. 126: 138–147.

Ralphs, M., R. Creamer, D. Baucom, D. Gardner, S. Welsh, J. Graham, C. Hart, D. Cook & B. Stegelmeier. 2008. Relationship between the endophyte *Embellisia* spp. and the toxic alkaloid swainsonine in major locoweed species (*Astragalus* and *Oxytropis*). J. Chem. Ecol. 34: 32–38.

Sampson, K. 1933. The systemic infection of grasses by *Epichloë typhina* (Pers.) Tul. Trans. Brit. Mycol. Soc. 18: 30–47.

Schardl, C. L. 2001. *Epichloë festucae* and related mutualistic symbionts of grasses. Fung. Genet. Biol. 33: 69–82.

——— & H.-F. Tsai. 1992. Molecular biology and evolution of the grass endophytes. Nat. Toxins 1: 171–184.

——— & F. Chen. 2010. Plant defences against herbivore attack. *In* Encyclopedia of Life Sciences. Macmillan Reference Ltd., Stockton Press, London, doi: 10.1002/9780470015902.a0001324, <http://onlinelibrary.wiley.com/doi/10.1002/9780470015902.a0001324.pub2/abstract>, accessed 22 September 2010.

———, A. Leuchtmann, H. F. Tsai, M. A. Collett, D. M. Watt & D. B. Scott. 1994. Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen, *Epichloë typhina*. Genetics 136: 1307–1317.

———, ———, K.-R. Chung, D. Penny & M. R. Siegel. 1997. Coevolution by common descent of fungal symbionts (*Epichloë* spp.) and grass hosts. Molec. Biol. Evol. 14: 133–143.

———, D. G. Panaccione & P. Tudzynski. 2006. Ergot alkaloids—Biology and molecular biology. Alkaloids 63: 45–86.

———, R. B. Grossman, P. Nagabhyru, J. R. Faulkner & U. P. Mallik. 2007. Loline alkaloids: Currencies of mutualism. Phytochemistry 68: 980–996.

———, K. D. Craven, S. Speakman, A. Stromberg, A. Lindstrom & R. Yoshida. 2008. A novel test for host-symbiont codivergence indicates ancient origin of fungal endophytes in grasses. Syst. Biol. 57: 483–498.

Selosse, M. A. & C. L. Schardl. 2007. Fungal endophytes of grasses: Hybrids rescued by vertical transmission? An evolutionary perspective. New Phytol. 173: 452–458.

Siegel, M. R., G. C. M. Latch, L. P. Bush, F. F. Fannin, D. D. Rowan, B. A. Tapper, C. W. Bacon & M. C. Johnson. 1990. Fungal endophyte-infected grasses: Alkaloid accumulation and aphid response. J. Chem. Ecol. 16: 3301–3315.

Soreng, R. J. & J. I. Davis. 1998. Phylogenetics and character evolution in the grass family (Poaceae): Simultaneous analysis of morphological and chloroplast DNA restriction site character sets. Bot. Rev. 64: 1–85.

Spatafora, J. W., G. H. Sung, J. M. Sung, N. L. Hywel-Jones & J. F. White. 2007. Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. Molec. Ecol. 16: 1701–1711.

Steiner, U., M. A. Ahimsa-Müller, A. Markert, S. Kucht, J. Groß, N. Kauf, M. Kuzma, M. Zych, M. Lamshöft, M. Furmanowa, V. Knoop, C. Drewke & E. Leistner. 2006. Molecular characterization of a seed transmitted clavicipitaceous fungus occurring on dicotyledoneous plants (Convolvulaceae). Planta 224: 533–544.

Sullivan, R., M. S. Bergen, R. Patel, G. F. Bills, S. C. Alderman, J. W. Spatafora & J. F. White. 2001. Features and phylogenetic status of an enigmatic clavicipitalean fungus *Neoclaviceps monostipa* gen. et sp nov. Mycologia 93: 90–99.

Sullivan, R. F., G. F. Bills, N. L. Hywel-Jones & J. F. White Jr. 2000. *Hyperdermium*: A new clavicipitalean genus for some tropical epibionts of dicotyledonous plants. Mycologia 92: 908–918.

Sullivan, T. J. & S. H. Faeth. 2008. Local adaptation in *Festuca arizonica* infected by hybrid and nonhybrid *Neotyphodium* endophytes. Microbial Ecol. 55: 697–704.

Sung, G. H., N. L. Hywel-Jones, J. M. Sung, J. J. Luangsa-ard, B. Shrestha & J. W. Spatafora. 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Stud. Mycol. 57(1): 5–59.

Tadych, M., M. Bergen, F. M. Dugan & J. F. White. 2007. Evaluation of the potential role of water in spread of conidia of the *Neotyphodium* endophyte of *Poa ampla*. Mycol. Res. 111: 466–472.

Tanaka, A., B. A. Tapper, A. Popay, E. J. Parker & B. Scott. 2005. A symbiosis expressed non-ribosomal peptide synthetase from a mutualistic fungal endophyte of perennial ryegrass confers protection to the symbiotum from insect herbivory. Molec. Microbiol. 57: 1036–1050.

Tanaka, E. & C. Tanaka. 2008. Phylogenetic study of clavicipitaceous fungi using acetaldehyde dehydrogenase gene sequences. Mycoscience 49: 115–125.

———, ———, A. Gafur & M. Tsuda. 2002. *Heteroepichloë*, gen. nov. (Clavicipitaceae; Ascomycotina) on bamboo plants in East Asia. Mycoscience 43: 87–93.

TePaske, M. R., R. G. Powell & S. L. Clement. 1993. Analyses of selected endophyte-infected grasses for the presence of loline-type and ergot-type alkaloids. J. Agric. Food Chem. 41: 2299–2303.

Timper, P., R. N. Gates & J. H. Bouton. 2005. Response of *Pratylenchus* spp. in tall fescue infected with different strains of the fungal endophyte *Neotyphodium coenophialum*. Nematology 7: 105–110.

Tsai, H. F., J. S. Liu, C. Staben, M. J. Christensen, G. Latch, M. R. Siegel & C. L. Schardl. 1994. Evolutionary diversification of fungal endophytes of tall fescue grass by hybridization with *Epichloë* species. Proc. Natl. Acad. Sci. U.S.A. 91: 2542–2546.

White, J. F. Jr. 1993. Endophyte-host associations in grasses. XIX. A systematic study of some sympatric species of *Epichloë* in England. Mycologia 85: 444–455.

——— & P. V. Reddy. 1998. Examination of structure and molecular phylogenetic relationships of some graminicolous symbionts in genera *Epichloë* and *Parepichloë*. Mycologia 90: 226–234.

———, A. C. Morrow, G. Morgan-Jones & D. A. Chambless. 1991. Endophyte-host associations in forage grasses. XIV. Primary stromata formation and seed transmission in *Epichloë typhina*: Developmental and regulatory aspects. Mycologia 83: 72–81.

Wilkinson, H. H., M. R. Siegel, J. D. Blankenship, A. C. Mallory, L. P. Bush & C. L. Schardl. 2000. Contribution of fungal loline alkaloids to protection from aphids in a grass-endophyte mutualism. Molec. Pl.-Microbe Interact. 13: 1027–1033.

Wille, P. A., R. A. Aeschbacher & T. Boller. 1999. Distribution of fungal endophyte genotypes in doubly infected host grasses. Plant J. 18: 349–358.

Yan, K., J. Yanling, S. Xianghui, Z. Lihui, L. Wei, Y. Hanshou & W. Zhiwei. 2009. Taxonomy of *Neotyphodium* endophytes of Chinese native *Roegneria* plants. Mycologia 101: 211–219.

Young, C. A., B. A. Tapper, K. May, C. D. Moon, C. L. Schardl & B. Scott. 2009. Indole-diterpene biosynthetic capability of *Epichloë* endophytes as predicted by *ltm* gene analysis. Appl. Environm. Microbiol. 75: 2200–2211.